

REPORT
ON
OBSERVATIONS MADE UPON THE CATTLE

AT
THE EXPERIMENTAL STATION AT OUTREMONT, P. Q.

RECOGNIZED TO BE TUBERCULOUS BY THE
TUBERCULIN TEST.

BY
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REPORT TO THE HON. SYDNEY FISHER, MINISTER OF AGRICULTURE,
OTTAWA, ON OBSERVATIONS MADE UPON THE CATTLE
AT THE EXPERIMENTAL FARM AT
OUTREMONT, QUE.

SIR,—We have the honour to forward to you the accompanying Report upon the observations made by us upon the cattle recognized as being tuberculous by the Tuberculin Test and transported from Ottawa, at the end of November, 1897.

These cattle, according to the reports forwarded to us from Ottawa, reacted so definitely to the Tuberculin Test that we, before beginning our work, expected that they would afford a most valuable object lesson upon the development and the dangers attendant upon the presence of this disease in cattle. This they have, although not wholly in the way we anticipated. A study of the animals extending now over nine months has indeed revealed many points which are most instructive and valuable, but, while our study has confirmed the observations of others upon the infectious nature of the disease and more especially upon the possible infectious quality of the milk and of the tissues of the animals, what it has equally shown us is that the milk of an animal suffering from extensive tubercular lesions, is by no means continuously infectious. This study has demonstrated that the tubercle bacilli may be found in the milk of diseased animals in which upon post mortem examination we have been unable to detect a single tubercular focus in the udders, and at the same time, our observations would seem most clearly to indicate that when an animal suffering from the disease, is kept in proper hygienic conditions, is well fed, and kept during the winter in a byre in which the ventilation and lighting are adequate, the disease may be kept at a standstill, or may even undergo a temporary arrest. While under these conditions, tubercle bacilli may be, by adequate methods, discovered in the milk, yet the number of these may be so small that inoculation of the milk in relatively large quantities (20 to 35 cc., or from $\frac{2}{3}$ of an ounce to somewhat over an ounce) into one of the most susceptible of animals, namely, the guinea pig, may be without effect.

We have during the course of the work elaborated and in the main employed a method for the detection of tubercle bacilli in milk and other fluids, which is simple and in our hands has given excellent results. A copy of the paper by Mr. E. W. Hammond, who has been working under us, detailing the method, is herewith inclosed. By the use of this method, it is, that we have reached this conclusion that bacilli may be detected in milk even when in such small quantities that inoculation with that milk will not affect susceptible animals.

At first sight, it may appear that the results obtained by us so far, are calculated to lessen the apprehension with which bovine tuberculosis is to be regarded, and are at variance with the observations of other workers. But others have also reported numerous cases in which the milk of animals reacting to Tuberculin has been found innocuous to the lower animals. Others again have found the tubercle bacilli in milk in cases in which they have been unable to detect any lesions in the udders. As regards the dangers attendant upon the use of the milk of tuberculous cattle, it must clearly be understood that our researches show that such milk is far from being constantly harmless; on the contrary, they indicate that, while for long periods in the earlier stages of the disease, that milk may either be free from bacilli, or contain so few as to be innocuous, at times, without any obvious change in the animal, so many bacilli may be passed out with the milk, that this becomes a most dangerous product. Indeed, the lesson taught is the very opposite, and is, that even if an animal affected with tuberculosis appears to be in a condition in which the disease is stationary, bacilli may appear in milk in varying numbers, and inasmuch as no one outside a laboratory would dream of making a weekly examination of samples of the milk of an individual cow, the only proper course to pursue is to prohibit the consumption of that milk.

In addition, our work, so far as it goes, showing as it does that the cattle if kept in good condition may retain their health for long months, the process apparently being

brought to a standstill, as again that calves may be fed for months upon their milk without tuberculosis being induced, would certainly seem to favour Professor Bang's contention, that tuberculous cows in which the disease is in an early stage, may well be kept for breeding purposes if separated from the non-tuberculous portion of the herd.

It will be seen from the following pages that our observations during the earlier months, have been mainly confined to Cow No. 1. It was this animal in which with more faulty methods of detection, we first discovered the tubercle bacilli in the milk and in which, with better methods, we found them regularly. The animal further had enlarged tubercular lymph glands at the base of the udder, whereas in none of the others could we detect any possible tubercular foci in the mammary glands by manual examination. The animal further reacted consistently to tuberculin.

In all these respects Cow No. 1, appeared to present more positive signs of advanced tuberculosis than any of the other animals, and we cannot but hold that we did wisely in selecting it for a first study. Even then it was months before we obtained satisfactory evidence of the infectious nature of her milk. Until we obtained these satisfactory results it appeared to us inadvisable to make extended observations upon other animals in which tuberculosis was less marked. As a result, Cow No. 1, afforded a field of more interesting study of many of the problems in connection with milk infection than perhaps any single animal has as yet afforded. Our series of observations of this one case may now be regarded as complete; in the others there is still work to be done. Hence in this report, we deal more especially with the results obtained from the study of this one animal.

Before however detailing the observations made by us upon the series of ten cows in general, or upon Cow No. 1 in particular, it may be well that we should state in general terms the nature of the observations made by us both for the detection of tuberculosis in the animals and of the tubercle bacilli in the milk, and again for the study of the process of infection more especially from the milk.

In order to detect the existence of tuberculosis, it is generally recognized that there are four main methods which may be employed. First and foremost, there is to be mentioned the Tuberculin Test, which reveals the existence of the disease more constantly than does any other method. Secondly, the recognition of the tubercle bacilli in the secretions and, in the case of the cow, in the milk. Thirdly, the reproduction of the characteristic lesions of the disease in animals by inoculation with either the diseased tissue or with the secretions; or again by feeding animals upon milk or other secretion. And last, and most absolute, the recognition of the specific lesions and bacilli of the disease at the post mortem examination.

Compared with these methods, the recognition of the disease in cattle by physical examination, save in very advanced cases, is of very dubious value and secondary importance. In our researches upon the Outremont cattle we have employed throughout all these methods and, as our conclusions will show, we believe we have obtained results which are of value in connection with the employment of all three methods. We propose therefore in the first part of our report to deal with the work accomplished by us in connection with the whole series of animals along each of these lines. By so doing, we shall give a resumé of the main mass of our observations. In the second part of our report we shall confine ourself to a history of Cow No. 1 and of the observations made in connection with that animal.

It may be that many of those interested in the work of your department are unacquainted with the amount of labour necessitated in observations such as those we have made, and as a consequence they may regard the accompanying report as giving but a small return in the matter of labour for the expenditure on the part of the government. We think it but right to point out that when the simple statement is made, for example, that liver, lungs, kidneys, and other organs, of the inoculated animal were examined, that statement alone indicates many hours work. The examination of a single inoculated animal involves the hardening and cutting of numerous sections from each organ; furthermore, such sections require not only the usual staining for the examination of tissues, but often, likewise, special methods for the detection of tubercle bacilli and often a prolonged hunt for the detection of such germs. Thus, it will be seen that the study of a large series of such inoculated animals involves, to say the least

not a little expenditure of time and energy. So again, the conscientious examination of a sample of milk takes so much time that only two samples can satisfactorily be studied in the course of one morning. The fewer the bacilli the greater the care and the longer the time necessary for the study of a given case. Thus as most of the samples of milk gave negative results and as the majority of the inoculated animals showed no signs of disease, progress in these investigations was, of necessity peculiarly slow.

In addition to the work which we ourselves have accomplished, we have found it advisable to engage the services of two other assistants whose time has to no small extent been devoted to this special line of work in your department. These assistants, we have ourselves appointed and employed without calling upon the government for their maintenance.

We have the honour to be, sir, your obedient servants,

J. GEORGE ADAMI,
C. F. MARTIN.

To the Honourable
The Minister of Agriculture,
Ottawa.

REPORT BY DR. J. G. ADAMI, M.A., M.D., F.R.S.E., PROFESSOR OF
PATHOLOGY, MCGILL UNIVERSITY, AND C. F. MARTIN, B.A. M.D.,
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On the 30th November, 1897, ten cows were received at the farm at Outremont from Ottawa. All these animals, which will hereinafter be spoken of as under the numbers 1 to 10, had been tested with tuberculin at the Experimental Farm, Ottawa, upon the 4th and 5th November, 1897, and all had reacted. In none of these animals, with the exception of cow 1, was there any recognizable evidence of tuberculosis; this cow suffered from a cough, and was in a rather poor condition, in addition, above and slightly in front of the udder two masses could be felt, firm and isolated and apparently in connection with the front portion of the mammary gland tissue; in Dr. McEachran's opinion these masses were of tuberculous nature. In the udder of cow 3, nodules were also to be felt, and these at first were diagnosed as tuberculous, but upon excision one of us (C. F. M.) found them to be of the nature of milk or retention cysts.

HOUSING AND MANAGEMENT OF THE CATTLE.

These ten cows were placed each in a separate stall in a byre newly built for the purpose, which was well lighted and at the same time warm and well ventilated. They were bedded upon dried peat covered by a layer of straw frequently changed. There was abundant supply of good spring water from a pump situated in the byre itself. Each cow had its own set of milking pails, &c. The partitions between the stalls were solid and of such a height (5 feet) that there could be no contact between the animals, and further, the ground in the neighbourhood of the byre had been fenced off so that each animal might be exercised and kept in the open by itself.

Opening into the byre in which these tuberculous animals were kept, was the store and fodder room in which was also a small furnace for warming the byre and for sterilising apparatus and preparing food for guinea pigs, &c. Opening out of this again was a small office for keeping the records and instruments of precision. Leading out of the store-room was a shed or byre about the same size as that in which the tuberculous cows were kept: here were stalls for calves and huts or pens capable of holding about fifty rabbits and guinea pigs. The history of each of the calves was ascertained, and only those animals whose parents had been free from the disease were accepted. Each of

the seven calves had its own drinking pail, and so far as possible were fed from the milk of one cow. As will be readily understood, the cows varied in the amount of milk which they gave, and some of them became dry, hence it was not possible to keep the whole of the calves fed upon the milk of one cow.

Every precaution was taken to isolate the diseased from the affected animals, and our results would appear so far to indicate that those precautions were adequate. So also the inoculated rabbits and guinea pigs have been kept isolated from the healthy uninoculated animals.

METHODS OF STUDY.

As already stated in the introduction to this report, the main observations made upon these tuberculous cattle, have been the following :—

1. Repetition of the Tuberculin test.
2. Attempted detection of the bacilli of tuberculosis in the milk of the suspected animals.
3. Investigations into the effect of inoculating milk from these animals into the very susceptible guinea pig and rabbit.
4. The effect of feeding calves with the milk of the suspected animals.
5. The post mortem examination of the cows.

TUBERCULIN TEST.

Upon the 16th of December, two weeks after the arrival of the animals at Outremont, and six weeks after the test of the same animals at Ottawa, seven of the cows, namely: Nos. 1, 2, 3, 4, 6, 7 and 8 were prepared for the test and their temperatures duly taken.*

Upon the following day the tuberculin employed by the Government, which has throughout given satisfactory results elsewhere, was injected into each of these seven animals by Prof. M. C. Baker, of Montreal. Of the seven cows, only two (Nos. 1 and 4) gave definite reaction. Cow 7 showed a definite rise of a little over 1° , but this rise could scarcely be spoken of as a reaction. Its highest temperature on the previous day had been 103° , and throughout that day it had averaged about 102.5° ; upon the morning following inoculation the temperature went up to 104° .

Temperature of cow No. 1 rose to 106° , a rise of 4.7°

“ “ No. 4 “ 104.2° “ 3.1°

“ “ No. 7 “ 104° “ 1.0°

It will thus be seen that six weeks after the first test only two of the animals gave a definite reaction. With a view towards ascertaining how soon a reaction might again be obtained, the cows were re-inoculated by Dr. Baker on 5th January, 1898 (20 days later); 60 mm. of tuberculin were employed and every animal was inoculated. None of the cows reacted. We learnt subsequently that the tuberculin employed, that of Parke, Davis & Co., had been kept for about two years, and then not always in a cool place, hence its powers must naturally be considered as having been somewhat dubious. We need scarce add that its failure to act under such circumstances does not in the slightest reflect upon the makers. This sample of tuberculin was employed owing to a misunderstanding.

On 15th January, Dr. Baker again inoculated the animals, employing the Government tuberculin with all due precautions, and of the ten, only three reacted, Nos. 1, 5 and 9; the temperature of 1 and 5 rising 4° , and of 9, 4.7° . It will be seen from this that two of the cows, 4 and 7 (which showed a rise of temperature upon 16th December), now failed to give any typical response; that two other cows, 3 and 9 not then tested, gave a reaction, and that only one cow, cow 1, reacted both times. We may here call to mind that this was the animals which presented the clearest evidence of tuberculosis. In

* The three other animals, 5, 9 and 10, were not tested, inasmuch as the attendant at the time was single handed and explained that he was too fully engaged upon the farm to take the temperatures of the preceding 12 hours of these animals.

connection with this animal it is to be noticed that a very definite reaction was obtained *within thirty days*, although it has been laid down by some authorities that within this period no second reaction is to be obtained in the cow. The cows were not inoculated again until the end of April, and now the effort was made to ascertain whether by increasing the quantity of tuberculin injected and giving very large doses, reactions might not follow sooner than is usual after previous injections. That is to say, the effort was made to inject unusually large doses of the tuberculin and thus to obtain a reaction. Such occurs in general in the human being in whom it is found that if, for example 1 mm. of tuberculin has caused a reaction, a week later the same amount will give no results, but if 2 mm. be employed, a definite reaction occurs.

For this purpose cows No. 4 and 7 were employed; their temperatures were taken upon 27th April; on the 28th, 120 mm. (double the usual quantity) of tuberculin were injected without effect. On 2nd May, after having again previously taken the temperatures, 240 mm. of tuberculin were injected into the same cows, but again there was no sign of reaction. Now, both of these cows had reacted at Ottawa in November, and one of them (4) had reacted in December, while the other (7) had then given a dubious reaction. Neither of them had reacted to 60 mm. of tuberculin in January; neither reacted to 120, 100 days later, or to 240, five days later again. It would seem that in this respect tuberculin acts somewhat differently in cattle to what it does in the human being. In connection with these animals it is worth noting that very possibly in them these large amounts and repeated injections of tuberculin had a curative effect—for, as will be seen in the section upon post mortem appearances, these two cows at their death, showed but a trace of tuberculosis.

We had been especially interested in this matter because, unfortunately, the fact that one dose of tuberculin in the cow nullifies a reaction of a similar dose within thirty days, has already been taken advantage of by the unscrupulous to conceal the fact that animals in their possession suffer from disease. Thus, not only may diseased animals be conveyed from one part of the country to the other, but even, they may escape detection by the inspectors when imported from other countries, and it appeared to us that possibly detection in these cases might be accomplished by the employment of massive doses of tuberculin.

So far as they go, our observations show that there is little hope of defeating the unscrupulous along these lines, and although our experiments are few, nevertheless, the results would seem to be positive, so positive that we have considered it unnecessary to make further research along these lines.

THE DETECTION OF TUBERCLE BACILLI IN MILK.

In order to carry out a regular examination of the milk of the animals, bottles of cylindrical shape, containing about 8 ounces were obtained, such as are employed for sterilising milk for infants. This form, having no angles, can be more easily kept clean and sterilised than the ordinary bottle; in place of corks, sterilised cotton wool was used to close them. A separate metal funnel sterilised before being used (by immersion in boiling water), was employed for each animal and the morning milk collected thus through the funnels into the bottles, either from a single quarter of the udder, or from all the quarters, was immediately brought to the laboratory. Here, in order to prevent the fermentation of the milk and in order to aid in the detection of the bacilli $2\frac{1}{2}$ per cent of glacial carbolic acid was added, and the bottles were placed in a cool cupboard to be examined in rotation.

A series of investigations were made as to how most surely to detect the bacilli in this milk.

At first, a long series of trials were made to observe the effect of mere sedimentation. After the milk had remained absolutely at rest for several days, by means of a pipette the sediment, which should contain any bacilli present in the sterilised milk, was drawn off and drops of this sediment were placed upon from five to ten separate clean glass slides and spread over the surface so as to form thin films. These films were evaporated fixed and treated with a mixture of alcohol and ether to remove the fat, and

in some cases after this with 10 per cent acetic acid to further dissolve out the proteid matters present, and were then stained in the usual way with carbol fuchsin and counter-stained by Gabbett's method. The milk from cows, 1, 3, 4, 6 and 8, were used in these earlier investigations. In not a single one of the samples from any of these animals, could we certainly detect any bacilli. Once or twice, isolated forms were seen which may have been bacilli but were not quite typical.

In another series of investigations extending over the early part of January, the milk was centrifugalised daily, at the rate of about 3,000 revolutions per minute. The sediment was obtained and treated in the way above mentioned, films were made, the fats dissolved out by alcohol and ether, &c. This method again gave uncertain results.

In a further series of cases, glacial acetic acid was added to milk up to the point of beginning coagulation, in the expectation that, as the casein, was thrown down, it would carry with it any tubercle bacilli present. We found it difficult, employing this casein, to obtain clear preparations; fine granules of the casein formed a very disturbing element in the picture making it difficult to be certain with regard to the outlines of possible bacilli.

In another series of observations, casein obtained as above, was hardened in alcohol and small portions imbedded in celloidin, fine sections of the imbedded and hardened mass being cut by the microtome. Here again the sections gave a rather blurred picture. In this casein series, however, out of eight different samples from the milk of cow No. 1, in two cases what we felt assured were tubercle bacilli were seen.

A further series of observations were made of centrifugalised cream, milk and sediment from these cows, and all at this early period without result, although when we added minute quantities of a suspension of the tubercle bacilli to fresh milk, and then centrifugalised, we had no difficulty in detecting the bacilli both in the sediment and in the cream.

Eventually, we devised a method described by Mr. Hammond in the accompanying pamphlet and by this means, we have since February obtained far more definite results. Whereas previously we had occasionally detected bacilli in the milk of cow No. 1, now, during March, we began to find them frequently, although it is true in very small numbers, and later, in May and June, we came across them in every sample of the milk although always in small numbers. By this same method we have occasionally met with bacilli in the milk of the other animals.

Before giving a classification of our results it is necessary that we should state our procedure with reference to the different animals. During December and the early part of January we studied the milk of cow No. 1, succeeded by the milks of cows, Nos. 3, 4, 6 and 8. In February we began the study of a week's milk from all the cows; the time taken for this brought us well into March. Similarly, all the milk of the 3rd week in March was submitted to examination. This work, as already stated in our letter of introduction, takes of necessity so long a time that during April and May, there being much of the March milk (carbolised) still unstudied, occasional samples of milk from cow No. 1 on the one hand and from those cattle which had given negative results, only were examined.

In June and July, the examination of the March milk from cow No. 6 having shown a sudden great increase in the number of bacilli, study of her milk was especially undertaken, samples of the other animals' milk being also under review but not studied with the same regularity. In August, was made an examination of the milk from Nos. 4, 6, 8 and 10 from the 20th to the 29th inst., and for the first week in September the milk from Nos. 2, 4, 6, 7, 8 and 10, were taken and examined daily.

Thus to epitomise, while occasional samples of the morning milk from all the cows have been examined each month, the routine examination of samples of successive days during the various months has been devoted to the milk of special cows. It would, we admit, have made our report more valuable had we been able to examine daily throughout the whole period a sample of the morning milk of each of the cows, but as already stated the time taken for the conscientious examination of a given sample, when that sample contains no bacilli or a very small number, is so long that with the assistance at our disposal, this has been an impossibility. Thus where we have found bacilli we have made routine daily examinations for several weeks at a time; where we did not find them in

the earlier samples, we examined the milk from time to time to observe whether they had made their appearance.

COW No. I.—Bacilli found occasionally in January milk, more frequent in February. Towards end of February, constantly found in milk about 15 in the field. From now on, through the samples of March, April and June milk examined, regularly found.

COW No. II.—Doubtful, and if present only very rarely and irregularly, the milk became very scanty and watery in August, but still free from bacilli.

COW No. III.—Rare beaded forms recognised in milk of February 14th, undoubted bacilli in milk of March 24th; in that of March 26th, again absent.

COW No. IV.—Three different samples of February milk showed bacilli, rare, one to three upon the slide. A few were found in the milk of March 24th as again in that of March 26th, immediately before and after these dates they were absent. Throughout May and June, not found, nor again in July milk. A few were found in milk of August 4th, after this they again disappeared. Present again upon August 24th, 25th, 27th and again in milk of week beginning September 3rd.

COW No. V.—Previous to March milk steadily decreased in bulk becoming yellowish and viscid until the animal became quite dry. The viscid milk contained very numerous cocci. Early in February doubtful bacilli were occasionally encountered. Throughout the latter end of February and beginning of March the fluid contained no bacilli.

COW No. VI.—Rare bacilli found in latter end of January; throughout February and early part of March absent. In milk of March 26th found in very great numbers. From this time on, not seen again till June 29th when a few doubtful forms were seen, the next day's milk showed none. From July 4th onwards until August 9th the bacilli were found in the majority of samples examined; upon August 23rd, again found, absent on August 24th.

COW No. VII.—One doubtful specimen in January; since then the milk has been constantly free from bacilli.

COW No. VIII.—A few bacilli of March 24th milk; none found at any other time.

COW No. IX.—None found previous to death in March.

COW No. X.—A small number of bacilli found in the August and September milks with fair regularity.

RESULT OF EXAMINATIONS.

It will be seen from the above table that in only two milks, even when these milks were concentrated by centrifugalisation, were bacilli found in any considerable numbers. In one of these, cow No. 1, bacilli were eventually found constantly, in the other, No. 6, they were apparently absent for several weeks during February and March and found constantly at the end of March; in one sample they were found in very great numbers. They again disappeared till the end of June, when a few dubious forms were seen; throughout July they were frequently found, disappeared again and were found on one occasion in August.

Cows No. 2, 5, 7 and 9 through the series of either showed no bacilli at all or rare very doubtful forms which we cannot term typical and certain bacilli. The other animals, namely, cows No. 3, 4, 8 and 10 at no time showed any large number of bacilli but on rare occasions presented forms which we were compelled to describe as undoubted bacilli. The variation in these four milks is of interest because when the bacilli were present they were unassociated with any recognizable change in the condition of the animal.

It is, further, of very considerable interest to notice that the two animals in which we found the greatest number of bacilli were those which, according to the findings at the autopsies performed upon them, (which see later) presented the most extensive condition of tuberculosis more especially of the lungs. Those in which we found no bacilli or very few, presented the least extensive disease. This fact is of importance as it is of strong support to our conclusions that what we saw were truly tubercle bacilli. It must be remembered that there has been not a little doubt on the part of some observers as to whether certain bodies found in the milk are truly tubercle bacilli. We had ourselves for a long period very considerable hesitation in arriving at the conclusion as to whether we were right in recognizing some of these bodies as tubercle bacilli.

In the first place, it may be laid down as to the tubercle bacillus that when seen in the milk it is considerably shorter and stumper than the bacillus one finds in the human sputum, for example, and what is more, is in general shorter than the form recognized in sections from the affected lung of the animal. When in an examination of milk one comes across certain minute bodies a little shorter than usual which nevertheless stain well by carbol fuchsin and are not decolorised by acid, it is extremely difficult to make up one's mind whether these bodies truly are tubercle bacilli. Where we recognized only these forms we spoke of them as doubtful forms. We must further remember also that just as in the examination of human urine for tubercle bacilli one may be confused by the presence of a somewhat similar form, the so-called smegma bacillus, so occasionally smegma bacilli or allied forms may make their appearance in the cow's milk. These smegma bacilli decolorise with greater ease than do the tubercle bacilli and we from time to time made tests which showed us that where we (J.G.A. and C.F.M.) disagreed as to the nature of the bodies, the reaction of these towards absolute alcohol after staining was that of the tubercle bacillus and not of the smegma bacillus.

I may here especially refer to the milk of cow No. 6, in which the sample of March 26th, when centrifuged, showed the bacilli in numbers far in excess of anything either of us had previously come across. But, the fact that these bacilli were strongly resistant both to acid and to alcohol, forced us to conclude that they must be tubercle bacilli. Accepting therefore that our diagnosis was correct, we next have to take into consideration the very remarkable fact, that while we found undoubted tubercle bacilli at one time or another in the milk of no less than 6 cows out of the 10 (1, 3, 4, 6, 8 and 10), in the post-mortem examination of the udders of all the ten cows we have, after careful examination, found not the slightest trace of tuberculosis of those organs. Had this been the case in connection with one cow only, or had we for example found that two of these animals presented tuberculosis of the udder or teats and the other two did not, then it might have been urged that the examination of the udders had been imperfect and that the presence of tubercle bacilli in the milk was due to mammary disease. But under the circumstances I think we are bound to conclude that tubercle bacilli can pass out through the mammary gland and can be excreted with the milk in the absence of any localised foci of disease in these organs. In short, we must conclude that the mammary gland like the kidney, and as one of us has recently demonstrated the liver, may act as one of the means whereby pathogenic micro organisms are removed from the system. We must confess that this is a conclusion which we had not anticipated; we do not however see how to escape it.

It is quite possible that in escaping thus, the bacilli are acted on to some extent by the mammary tissue, and if this be so, such action may explain the somewhat altered shape of many of the bacilli and the fact that the milk of cow No. 1, while containing what would seem above the minimum number of bacilli which have been found necessary to infect a guinea pig or rabbit rarely caused such infection.

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We should here say that our method of examination of the mammary glands for the existence of tubercles, was as follows: At the autopsy each udder was cut into a series of thin slices and carefully examined throughout and each slice carefully examined under the naked eye. Special care was taken in the examination of the teats and the galactiferous ducts. Following upon this several portions of each gland were preserved, hardened and cut into sections, here again more special care was taken to obtain sections

from the larger milk tubes and galactiferous ducts, and as already stated, in not a single sections have we found, under the microscope, a sign of tubercle.

Under the naked eye, one udder—that of Cow No. 10—certainly appeared suspicious, whitish rather caseous tubercles being scattered in fair abundance through the mammary gland tissue; upon microscopical examination, however, without exception these nodules or tubercles were found to be, what had been suspected by us, localised areas in which the lobules of the gland tissue had through some obstruction become blocked up with inspissated milk.

While we did not find any recognisable tubercles it is worthy of note, that in the majority of these animals the udders were tougher and more fibroid than normal; this was noticeably the case in cows No. 1, 2, 3, and 6. It may be questioned whether this increased development of fibroid tissue throughout the gland had any possible relationship to the existence of the disease in other parts of the body. Where present it was fairly well generalised and not localised. We know that some authorities have in the cases of other organs, as for example the liver, suggested that interstitial fibrosis may follow the development of tuberculosis. Certainly in the case of the cow's udder, the fibrosis is so generalised, and the absence of anything like existing tubercles in our cases was so marked that, if there be any relationship between the tuberculosis and the interstitial fibroid state, we certainly cannot regard that relationship as brought about by pre-existing tubercle development in these glands. Further it is a well established fact that such interstitial fibroid change may be brought about by other conditions: previous inflammatory disturbances in the organ of various kinds might lead to such development, and as animals become aged there is a liability for the appearance of the same. In the animals studied by us it is true, we have no right to assume the previous existence of these conditions and we can only now sum up by saying that the result of our examinations has led us to believe that the condition of increased development of interstitial fibrous tissue was noticeable in the mammary glands of a fair proportion of the animals studied by us and suffering from tuberculosis. We must leave it an open question as to whether this was a mere coincidence or whether there is a direct connection. Again we must acknowledge at the present moment, if there be such connection, we are not in a position to explain the mode of development of the condition.

Thus our work so far tends to lead us to the conclusions:—

1. That tubercle bacilli may appear in the milk of cattle which are free from any evidence of tuberculosis of the mammary gland tissue.

2. That while this is the case, the number of bacilli appearing in the milk of these cattle is inconstant and in general very minute, so that even inoculating large amounts of this milk into the animals of the laboratory, no tuberculosis is necessarily set up in those animals. To these conclusions, we may add:

3. In such animals without any obvious change in the general conditions the number of bacilli passing into the milk may temporarily be increased greatly, so that by the very number present, the milk becomes a most dangerous product.

4. That, while the milk containing these small numbers of bacilli, in general sets up no tuberculosis when inoculated into the most susceptible of animals, in a certain small proportion of cases (to be described later), it does lead to the development of tuberculosis, hence that milk cannot be regarded as a safe product, and consequently—

5. Where an animal has reacted to tuberculin, the fresh unsterilised milk should never be used as a food stuff.

THE EFFECTS OF INOCULATING MILK FROM THESE ANIMALS INTO THE RABBIT AND GUINEA PIG.

The results of the work done by us along these lines is shown in the accompanying tables.

EFFECTS of Inoculating Milk from Animals

Number.	Date of Inoculation.	Cow Number.	Special Source of Milk.	Treatment of Milk before Inoculation.	Animal Employed	Site of Inoculation.	Weight and Temperature before Inoculation.	
							Grms.	Degrees.
	1897.							
1	Dec. 30	I	Right anterior quarter of udder	†2cc. of 1st milk of morning; centrifugalized.	Guinea pig	Intraperitoneal	790	101·4
2	" 30	I	4 quarters.....	16cc. used; centrifugalized.	" ..	" ..	650	101·
	1898.							
3	Jan. 6	I	Right anterior quarter.	Centrifugalized; 20cc. milk.	" ..	" ..	660	101·4
4	" 6	I	4 quarters....	20cc. used; centrifugalized.	" ..	" ..	785	101·4
5	" 9	I	Right anterior quarter.	*Casein particles.....	" ..	" ..	482	101·
6	" 9	I	" ..	Cream; 16cc. used....	" ..	"
7	" 9	I	" ..	16cc. skimmed milk of case No. 6.	" ..	" ..	600
8	" 18	I	4 quarters.....	Casein particles	" ..	" ..	632	101·5
9	" 18	IV & VI	Control.....	Mixed 6cc. of cream with culture of tubercle bacilli.	" ..	" ..	687	99·6
10	" 18	IV & VI	Control.....	Mixed 10cc. of skimmed milk with culture of tubercle bacilli.	" ..	" ..	672	101·8
11	" 21	I	4 quarters.....	Casein particles.....	" ..	" ..	537	99½
12	" 23	VI	" ..	Mixed milk 10cc. and cream.	" ..	" ..	352	99·2
13	" 23	IV	" ..	10cc. mixed milk and cream.	" ..	" ..	318	98·4
14	" 23	VIII	" ..	6cc. mixed milk and cream.	" ..	" ..	412	99·4
15	" 29	III	" ..	15cc. mixed milk and cream.	" ..	" ..	800	99·6
16	Feb. 24	I	Right anterior quarter.	16cc. milk; centrifugalized.	Rabbit ...	" ..	1793	102·1
17	" 24	I	4 quarters.....	18cc. cream.....	" ...	" ..	1144	101·4
18	" 24	IV	" ..	20cc.; bottled merely..	" ...	" ..	1459	102·4
19	April 1	I	" ..	15cc. " ..	" ...	" ..	1940	101·6
20	" 1	I	" ..	20cc. " ..	" ...	" ..	1957	101·2
21	" 1	I	" ..	20cc. " ..	" ...	" ..	1550	105·
22	" 1	I	" ..	25cc. " ..	" ...	" ..	1960	102·
23	" 3	I	" ..	20cc. cream	" ...	" ..	1765	100·2

* In all cases not otherwise mentioned the inoculated milk was taken from the first milk drawn off
† Casein was produced by adding 3 drops of glacial acetic acid to six ounces of milk.

into the Rabbit and Guinea Pig.

Ultimate Event with Dates.	Loss of Flesh since Inoculation.	Presence of Tuberculosis.	Result of Autopsies on Inoculated Animal.	Microscopic Examination of Tissues from Inoculated Animals.
Died Jan. 30, '98.	55 grammes ...	None.....	No tuberculosis found. Hemorrhages in lung tissue.	Sections of lung, liver, kidney and spleen examined. No evidence of tuberculosis.
Died Jan. 12, '98.	"	Tuberculosis absent.....	Sections from liver and spleen found normal.
Died Jan. 7, '98; ruptured stomach.	Stomach ruptured as result of inoculation experiment	
Died Jan. 31, '98.	285 grammes ...	None.....	No abnormality found. Cultures from organs remained sterile.	No abnormality in sections from the liver and lungs.
Lost
Died Feb. 2, '98.	None.....	No tuberculosis found. Cultures sterile.	Liver, lung, spleen and peritoneum examined, but all were normal.
Died Jan. 19, '98, of septic infection.	29 grammes ...	"	Subcutaneous phlegmon of trunk from operation wound.	
Died June 18, '98.	None.....	"	No abnormality found.....	Sections from kidney, liver, spleen and testis all found normal.
Died Feb. 2, '98.	Rapid	Marked and generalized tuberculosis.	Tubercles in peritoneum, liver, kidney, spleen, lungs. Lymph on peritoneum.	Sections confirmed microscopic diagnosis. Bacilli of tuberculosis in sections.
Died Feb. 14, '98.	"	"	Tubercles on peritoneum, in liver, spleen; also on intestine.	Sections of liver and spleen showed tubercles, with bacilli of tuberculosis.
Died Feb. 15, '98.	None.....	Doubtful spots in liver and spleen. Catarrhal enteritis, elsewhere nothing.	No tubercles found in sections of spleen or liver, and no bacilli after special staining.
Died June 24, '98.	Gained 4 grms.	"	Organs appear healthy. No cause of death assigned.	
Died June 29, '98.	" ..	"	Distended stomach, red peritoneum. No tuberculosis evident.	Spleen, liver and peritoneum examined. Early infiltr of peritoneum, but not tuberculosis.
Died Mar. 28, '98.	62 grammes ...	"	Doubtful nodules on spleen. Fibromata. liver healthy, also peritoneum.	No tubercles found in sections of liver, lungs or spleen. Lungs splenized.
Died Mar. 29, '98.	93 " ...	"	No evidence of tuberculosis.	Nothing in any of the sections.
Died April 6, '98.	Marked ..	"	2 gray nodules in liver, coccidia, elsewhere no disease.	Coccidia found in liver. No trace of tuberculosis in sections of organs.
Died Aug. 3, '98.	Lost much weight.	"	No sign of death causation.	No tuberculosis.
Lost	Error of servant at farm...	
Killed July 8, '98.	Gained 65 grms	None.....	No evidence of tuberculosis	Coccidia in liver.
Killed July 8, '98.	Lost 117 grms..	"	No sign of disease.....	
Sick prior to using; died.	" 240 "	Not employed till Sept., '98	
Killed July 8, '98.	" 90 " ..	None.....	Organs all healthy	
Killed July 8, '98.	" 225 " ..	"	"	

in the early morning, and was employed within 3 hours from the time of being obtained.

EFFECTS of Inoculating Milk from Animals

Number.	Date of Inoculation.	Cow Number.	Special Source of Milk.	Treatment of Milk before Inoculation.	Animal Employed	Site of Inoculation.	Weight and Temperature before Inoculation.	
							Grams.	Degrees
	1898.							
24	April 3	I	4 quarters.....	15cc. milk ; bottled....	Guinea pig	1507	102°
25	" 3	I	"	20cc. ; bottled	" ..	Intraperitoneal .	1890	104°
26	" 3	I	"	20cc. "	" ..	" ..	1000	103° 8
27	" 3	I	Control	" ..	Not inoculated ..	895	104° 8
28	" 7	I	Right anterior quarter.	10cc. milk ; bottled ...	" ..	Intraperitoneal .	575	101° 2
29	" 7	I	" ..	20cc. ; bottled.....	" ..	" ..	390	103° 2
30	" 7	I	"		
31	Apr. 7	I	4 quarters.....	— c.c., bottled.....	" ..	v. case No. 61...	615	103° 4
32	" 7	I	" ..	15 "	" ..	Intraperitoneal .	580	102° 4
33	" 13	I	" ..	15 "	" ..	" ..	665	102° 2
34	" 13	I	" ..	10 "	" ..	" ..	445	103°
35	" 14	I	" ..	20 "	" ..	" ..	660	102° 5
36	" 14	I	" ..	20 "	" ..	" ..	565	102° 2
37	" 29	I	" ..	20 "	" ..	" ..	670	101° 6
38	" 29	I	" ..	25 "	" ..	" ..	635	102° 4
39	" 29	I	" ..	30 "	" ..	" ..	665	103° 4
40	" 29	I	" ..	30 "	" ..	" ..	647	102°
41	" 29	I	" ..	18 "	" ..	" ..	635	103°
42	" 29	I	" ..	15 "	" ..	" ..	695	103°
43	" 29	I	" ..	20 "	" ..	" ..	642	101° 6
44	" 29	I	" ..	18 "	" ..	" ..	500	102° 4
45	" 29	I	" ..	15 "	" ..	" ..	589	101° 2
46	" 29	I	Control.....	No inoculation made..	"	307	101° 8
47	" 20	I	4 quarters.	25 c.c., bottled	Rabbit ..	Intraperitoneal .	2265	101°
48	" 20	I	"	25 "	" ..	" ..	1930	101° 4
49	" 20	I	" ..	30 "	" ..	" ..	2290	99°
50	" 20	I	" ..	25 "	" ..	" ..	1687	100° 8
51	" 20	I	" ..	35 "	" ..	" ..	2220	101° 6
52	" 20	I	" ..	25 "	" ..	" ..	1625	100° 4

into the Rabbit and Guinea Pig—*Continued.*

Ultimate Event and Dates.	Loss of Flesh since Inoculation.	Presence of Tuberculosis.	Result of Autopsies on Inoculated Animals.	Microscopical Examination of Tissues from Inoculated Animals.
Killed July 8, '98.	Lost 267 grms..	None.....	Organs all healthy.....	
Sick prior to using.	" 70 "	Not employed till Sept....	
Died June 18, '98.	" 40 " ..	None.....	No evidence of tuberculosis.	Coccidia found in liver.
Died June 18, '98.	"	"	
Killed Aug. 1, '98.	Gained	"	All organs healthy.....	
Died 40 hours later.	Septic infection from operation.	Coccidia in liver.
Sick already; not used.	Died before using.....	No tuberculosis.
Inocul. May 24 . v. case No. 61..	
Killed July 8, '98	Gained ..	None	Focal necrosis in liver, but no signs of tuberculosis.	Many sections cut and examined for bacilli, but none found in liver or lungs.
" 8, '98	Lost 5 grms ...	"	No evidence of disease.....	
" 5, '98	Gained	"	No evidence of disease except small nodule near ovary.	Spleen, liver, nodule near ovary examined, but no tuberculosis found.
" 5, '98	Lost 25 grms ..	"	No evidence of disease.....	Lung, fat near ovary, liver examined, also spleen; all normal.
Died May 29, '98	"	No sign of tuberculosis. Focal necrosis in liver and kidney.	Liver, spleen, kidneys and lungs examined; no signs of tuberculosis.
Killed July 6, '98	Lost 60 grms ..	"	No evidence of disease.....	Same organs; also a little nodule on the omentum, merely fibroid. No tuberculosis. Testicle and epidermis examined too, and found normal.
" 6, '98	" 55 " ..	"	"	
" 6, '98	" 15 " ..	"	"	Spleen and liver examined and found normal.
" 5, '98	" 57 " ..	"	No evidence of tuberculosis. Necroses in liver.	Sections of lung, liver and spleen made. No sign of tuberculosis; also cartilage of ribs; no disease.
" 6, '98	" 30 " ..	"	No evidence of disease.....	
" 6, '98	Gained	"	"	
" 6, '98	Lost 72 grms ..	"	Focal necroses in liver. No tuberculosis.	No signs of tuberculosis in sections from lungs or liver or spleen.
" 6, '98	Gained	"	No evidence of disease.....	
Died May 30, '98	Marked tuberculosis.	Tuberculosis peritonitis. Tubercles in liver, spleen, lungs and pleura.	Bacilli of tuberculosis found in sections of organs, with tubercles.
Killed July 6, '98	Gained ...	None	Organs appear healthy...	
" 11, '98	Lost 180 grms ..	"	"	
Died May 25, '98	" 30 " ..	"	Remarkable distension of stomach, with obstructed pylorus. No other disease.	Sections of liver and spleen; normal.
" 11, '98	" 180 " ..	"	Organs appear healthy...	
Killed Jly. 11, '98	" 72 " ..	"	Coccidia in liver. No tuberculous tissue anywhere.	Liver shows its parasites. No tuberculosis.
" 11, '98	" 410 " ..	"	Organs are healthy except for small spleen and necrosis in liver.	No tubercles in liver, spleen or lungs.
Killed Aug. 1, '98	Gained ..	"	Coccidia in liver. No tuberculous organs anywhere.	

EFFECTS of Inoculating Milk from Animals,

Number.	Date of Inoculation.	Cow Number.	Special Source of Milk.	Treatment of Milk before Inoculation.	Animal Employed	Site of Inoculation.	Weight and Temperature before Inoculation.	
							Grams.	Degrees.
1898.								
53	April 20	I	4 quarters.....	25cc. bottled.....	Rabbit ...	Intraperitoneal..	952	100·6
54	" 27	I	"	24 "	"	" ..	1142	102·
55	" 27	I	"	25 "	"	" ..	1227	101·6
56	" 27	I	"	35 "	"	" ..	600	101·8
57	" 27	I	"	25 "	"	" ..	640	103·2
58	" 27	I	"	50 "	"	" ..	1880	102·2
59	May 24	I	"	30 "	Guinea pig	" ..	687	102·6
60	" 24	I	"	20 "	"	" ..	352	102·4
61	May 25	I	"	35 "	Guinea pig	Intraperit	692	103·0
62	" 25	I	4 "	32cc. "	" ..	Intraperit. and subcutaneous.	527	103·0
63	" 25	I	4 "	50cc. "	" ..	" ..	517	102·8
64	" 25	I	4 "	24cc. "	" ..	Intraperit	537	103·2
65	" 25	I	Right anterior quarter.	24cc. "	" ..	Intraperit. and subcutaneous.	642	102·4
66	" 25	I	" ..	24cc. "	" ..	Intraperit	567	103·0
67	July 9	III	3 quarters.....	30cc. "	Rabbit ...	"	2100	103·4
68	" 9	III	3 "	30cc. "	"	"	1070	103·8
69	" 9	V	4 "	30cc. "	"	"	1262	103·2
70	" 9	V	4 "	60cc. "	"	Intraperit. and subcutaneous.	1492	104·2
71	" 14	IV	4 "	40cc. "	"	Intraperit	695	104·0
72	" 14	IV	4 "	40cc. "	"	"	1900	104·2
73	" 14	VI	4 "	40cc. "	"	"	1410	103·8
74	" 14	III	4 "	40cc. "	"	"	1345	104·0
75	" 16	V	4 "	40cc. "	Guinea pig	Intraperit. and subcutaneous.	700	102·6
76	" 16	V	4 "	40cc. "	" ..	Intraperit	402	103·0
77	" 16	VI	4 "	40cc. "	" ..	"	595	103·2
78	Aug. 5	V	4 "	20cc. "	Rabbit ...	"	1217
79	" 5	V	4 "	20cc. "	"	"	1387
80	" 5	V	4 "	20cc. "	"	"	1030	103·2
81	" 5	III	4 "	20cc. "	"	"	1150	103·2

*The numerous deaths occurring between Nov. 16th and 22nd were due to animals at Outremont

into the Rabbit and Guinea Pig—*Continued.*

Ultimate Event with Dates.	Loss of Flesh since Inoculation.	Presence of Tuberculosis.	Results of Autopsy on Inoculated Animals.	Microscopic Examination of Tissues from Inoculated Animals.
Killed Aug. 1, '98 Died July 21, '98	Gained " and lost.	None. "	Organs are healthy. Coccidia in liver. No other abnormality.	Liver, spleen, lungs examined. No tuberculosis.
Killed Aug. 1, '98 " 1, '98 Died May 27, '98	" " "	" " "	Organs healthy. " Marked coccidiosis. No tubercles to be seen anywhere.	Spleen, liver, small intestines and peritoneum examined and found normal apart from coccidia.
Killed Aug. 1, '98 " Sept. 2, '98 " Aug. 14, '98 Died Aug. 8, '98	Lost 35 grms. . Gained " Lost 147 grms..	" " " <i>Marked tuberculosis.</i>	Organs healthy. " " <i>Tuberculous peritoneum, lungs, pleura, liver and spleen.</i>	Tubercles in all mentioned organs and bacilli of tuberculosis found in the sections.
Killed Aug. 14, '98 " " 14, '98 " " 14, '98 " " 14, '98 " " 14, '98	Lost nothing. . Gained " " "	None. " " " "	Organs appear healthy. Doubtful nodules in spleen; all else is normal. Organs healthy. Whitish grey punctate spots in liver; other organs healthy. Spleen large and nodular; nothing else abnormal.	No abnormality in sections of lungs, liver and spleen. Spleen—fibroid patches. No tubercles. No tubercles in liver. No sign of tuberculosis in spleen kidneys, liver or peritoneum.
Died July 31, '98 " " 22, '98 " " 10, '98 " " 18, '98 " " 30, '98 * " Nov 21, '98 " July 15, '98 " " 22, '98 " " 22, '98 " Nov. 22, '98 " " 18, '98 Died. Not taken to laboratory. Died Aug. 31, '98 Died. Not taken to laboratory. Died Nov. 21, '98	Lost 700 grms. . Lost — grms. Lost 180 grms. Lost 320 grms. Lost 192 grms. . " 197 " Lost 387 grms. Lost 152 grms. .	" " None. " " None. None. None. None. None.	Whitish areas in liver; other organs normal. Died of pus infection. Died of shock from operation Cause of death doubtful—caecal appendix shows nodules on walls, but nothing else. Peritoneum contains what seems non-inflammatory fluids; organs seem O.K. No tuberculosis Post operative peritonitis .. Infection from inoculation wound and subcutaneous phlegmon. Organs healthy. Cause of death? Extensive emaciation with some hydremia. No tuberculosis No autopsy made. (Error of servant.) No sign of tuberculosis in any organs. No autopsy. (Error of servant.) No tuberculosis.	Coccidia in liver. No tuberculosis in any sections of spleen, liver and lungs. Lungs and spleen examined. No sign of tuberculosis. No tubercles in organs anywhere. No signs of tuberculosis in spleen, liver or lungs. " " Liver, spleen, skin and kidney examined. No tuberculosis found. Spleen and site of inoculation. No tuberculosis. No signs of tuberculosis. " " No sections made. No microscopical sign of disease.

not having been properly cared for during a sharp spell of cold weather.

EFFECTS OF Inoculating Milk from Animals,

Number.	Date of Inoculation.	Cow Number.	Special Source of Milk.	Treatment of Milk before Inoculation.	Animal Employed	Site of Inoculation.	Weight and Temperature before Inoculation.	
							Grams.	Degrees.
	1898.							
82	Aug. 5	III	4 quarters.	20cc. bottled.	Rabbit ...	Intraperit.	1245	103·4
83	" 17	VII	4 "	15cc. "	Guinea pig	"	293	102·2
84	" 17	VII	4 "	15cc. "	" ..	"	290	102·2
85	" 17	VII	4 "	15cc. "	" ..	"		
86	" 17	VI	4 "	15cc. "	" ..	Intraperit. and subcutaneous.	450	102·8
87	" 17	VI	4 "	15cc. "	" ..	Intraperit.	298	102·2
88	" 17	VI	4 "	30cc. "	" ..	Intraperit. and subcutaneous.	712	102·0
89	" 17	IV	4 "	20cc. "	Rabbit ...	Intraperit.	1208	102·6
90	" 17	IV	4 "	18cc. "	" ...	"	1177	103·4
91	" 17	IV	4 "	18cc. "	" ...	"	918	102·8

N. B.—Though this report is to the 31st October, 1898, it has been thought well to publish the result of these interesting experiments to the date of the temporary closing of the Outremont Experimental Station in the following month.

into the Rabbit and Guinea Pig—*Concluded.*

Ultimate Event with Dates.	Loss of Flesh since Inoculation.	Presence of Tuberculosis.	Results of Autopsy on Inoculated Animals.	Microscopic Examination of Tissues from Inoculated Animals.
Died Nov. 16, '98	Lost 453 grms.	<i>Marked tubercu- losis.</i>	<i>Very extensive, affecting every organ.</i>	Tubercles with bacilli in all organs.
" " 16, '98	Much emaciated	No tuberculosis	No microscopical evidence of tubercles or bacilli.
" " 21, '98	G'ned Sep. 1, '98	"	" "
Died Aug. 30, '98	No record.....	None.....	Organs show no tuberculosis anywhere.	" "
Died Oct. 19, '98	G'ned Sep. 1, '98	No tuberculosis	" "
Died Aug. 31, '98	Lost	None.....	No tuberculosis of organs..	" "
" Nov. 16, '98	G'ned Sep. 1, '98	No tuberculosis	" "
" " 22, '98	Lost 108 grms..	"	" "
" " 22, '98	" 93 "	"	" "
Living.....	G'ned Sep. 1, '98	"	" "

It will be seen then from the above details that there have been in all more than 90 animals employed and inoculated or used for the purposes of controls. Of these, 42 rabbits and 44 guinea pigs have been inoculated with milk from the tuberculous cows. Two guinea pigs for the purpose of control were inoculated with milk into which had been placed portions of the living cultures of tubercle bacilli and both of these animals rapidly succumbed to the disease.

Of all the animals inoculated with milk, only three have so far given evidence of tuberculosis, two of them being guinea pigs. This makes the average of guinea pigs inoculated with milk from the tuberculous cattle and developing the disease, as a little over 4 per cent. But one of the rabbits so far inoculated has become infected by the milk.

To state briefly the conditions under which the two guinea pigs died from the effects of the milk, it may be said that one died within four weeks, having received intraperitoneally 15 cc. of milk bottled in the usual way and consisting of the first milk drawn from cow 1 in the morning. The inoculation was made on April 29th and the animal died on May 30th, showing the usual evidence of tuberculosis in the peritoneum, spleen, liver and lung pleura. Examination further revealed in these diseased tissues the bacilli of tuberculosis. The second guinea pig died nine weeks after the primary inoculation, having received into its peritoneal cavity 35 cc. of bottled milk removed from all four quarters of the udder of cow No. 1 (the first morning milk). Here too the evidence of tuberculosis in the guinea pig were seen in the peritoneum, liver, spleen, lungs and pleura; the bacilli when looked for by the usual staining methods for that purpose were found in the affected tissues.

The solitary rabbit succumbing to infection was inoculated intraperitoneally upon August 5th, receiving 20 ccm. of milk from cow III. (It is deserving of note that the milk of this animal had showed on several occasions rare but definite bacilli and that at the autopsy the amount of tuberculous infection in it was found to be very limited.) In the three weeks after inoculation the animals gained slightly in weight. Later it showed progressive emaciation. While at the autopsy we found very extensive evidences of disease, with tubercles in all the important organs, the disease was of a subacute or chronic type; for death occurred some fourteen weeks after inoculation, and then appeared to be hastened by lack of adequate attention during a severe spell of cold weather. A companion rabbit, inoculated at the same time with like quantities of the same milk did not show a sign of tuberculosis when it died at the same period.

With reference to the inoculations in general, it may be here stated that for the most part the milk was removed from all four quarters of the udder and that first obtained in the morning was alone employed. In some cases, as in cow No. 1, where one particular quarter of the udder seemed more especially diseased, the milk was removed from that portion alone. In some cases the milk was centrifugalised, in others it was simply shaken up in the bottles in which it was received.

Again in some cases the cream alone was inoculated, in others the skimmed milk, but in the majority the unaltered milk was employed. The smallest amount inoculated in any one animal was 2 ccm. (of sediment from centrifugalised milk), the largest 60; and on the average about 20 ccm., that is $\frac{2}{3}$ of an ounce were employed. The casein alone was inoculated in some of the experiments in the hope that by adding glacial acetic acid to the milk and thereby precipitating the casein, it might be possible to find the bacilli more abundant where precipitated along with the particles of casein; so far, however, we have been unable to substantiate this supposition.

The weights and temperatures of the animals prior to inoculation were carefully ascertained, and from time to time the weighing was repeated. In the animals afflicted with tuberculosis, there was, as one would expect, marked loss of flesh, and in many others from no apparent cause a similar result ensued, though on the whole the majority of these inoculated with the milk from those tuberculous cattle seemed rather to gain than to lose flesh. It is an open question as to what is the cause of this not unfrequent emaciation of animals in which, although inoculated with material from tuberculous sources, tuberculosis does not supervene. I learn from Dr. Baldwin, of the Saranac Laboratory for the study of tuberculosis, that he and other workers under Dr. Trudeau, have frequently observed the same phenomenon.

Wherever animals died subsequent to inoculation, a careful autopsy was made in order to determine the possible presence of disease, and furthermore, those animals which survived the inoculation for any length of time greater than that in which tuberculosis presumably would have developed, were killed and autopsies made in order more satisfactorily to determine their condition. In nearly all the cases moreover, the autopsies were made more complete by microscopical examinations of the tissues and due notes taken of the abnormalities therein found.

The statistics may be briefly summarised as follows :—

Subsequent to inoculation,

One animal died one week after inoculation.

Five animals died two weeks after inoculation.

Five animals died three weeks after inoculation.

One animal died five weeks after inoculation.

Four animals died one month after inoculation.

Four animals died two months after inoculation.

Two animals died three months after inoculation.

Four animals died five months after inoculation.

Two were inoculated with milk into which had been placed artificially a culture of tubercle bacilli, and these both died within a few weeks. Four were not used, being kept as controls or because of their being apparently ill already. Seven died as a direct result of the experiment, either from rupture of the internal viscera or from septic infection and post operative peritonitis. There are four of which no record has been kept, due alone to the error of the servant in charge of the Farm, who neglected to report the deaths of these animals, thus obviating the possibility of a further examination, the animals being thereby disposed of before being seen by those in charge.

Thirty-five were killed when a sufficient time had elapsed to suppose that they might show lesions of tuberculosis, and 12 of those inoculated are still living *and we are now waiting further developments in their case, the time being not yet ripe for further examination of them.

From cow No. 1 alone, fifty-six animals were inoculated, both guinea pigs and rabbits. And as above stated two of the guinea pigs died from tuberculosis.

From cow No. 3, six animals were inoculated.

From cow No. 4, seven animals were inoculated.

From cow No. 5, seven animals were inoculated.

From cow No. 6, six animals were inoculated.

From cow No. 7, three animals were inoculated.

From cow No. 8, one animal was inoculated.

None of these have as yet shown any sign of tuberculosis though in many instances the time for development of tuberculosis is not complete and we therefore await further examination before giving a more detailed report of our results with these latter investigations.

FEEDING EXPERIMENTS.

Experiments have further been made with the milk from these tuberculous cows, to ascertain if from animals so lightly affected, one would obtain milk which would be infective to calves and for this purpose calves have been kept in separate stalls and have been fed from the milk obtained from these tuberculous cattle in the following manner :—

The milk from these cattle, not otherwise used for inoculation purposes, was drawn off from the individual cows, into separate thoroughly cleansed and sterilised pails in such a way that each calf, duly marked and isolated, was fed with milk from one cow alone and obtained during the time of the experiment absolutely no other food whatever ; at no time were these pails interchanged nor until the last three months when the killing of certain cows rendered it necessary, was the milk from one cow given to any other but the individual calf assigned for such feeding.

* NOTE.—In the appended tables, completed when these pages are in print, these 12 animals will be accounted for.

During the early periods of this experiment it was deemed necessary to inoculate with tuberculin those calves then in our possession, namely, 3 in number, and after duly taking the temperature, 25 mm. of tuberculin were injected into each and they in each case failed to react. This being the first ever injected into these animals, it was reasonable to conclude that no tuberculosis was present prior to the experiments performed; the tuberculin was injected on 5th December. The three calves mentioned, were none of them over two weeks of age. A fourth calf also received from Ottawa, was suspected of having tuberculosis, the animal was killed and an autopsy made but no tuberculosis was discovered.

Another calf was obtained from our own cow No. 5 on 5th June and the feeding here as before was maintained from its own mother. It was inoculated one month later, July 6th, and showed a failure to react and since that time no further injections of tuberculin have been made into these calves.

On 5th January, another calf was obtained from Mr. Ried, of Outremont, and prior to experiment was inoculated with tuberculin as was also the mother; in both cases, the cow and the calf, failed to react. This calf kept in a special stall was fed from the milk of cow No. 1, the most diseased of all our animals: tested with tuberculin on 31st January and again on 6th July it likewise gave no reaction.

Another calf obtained from our own cow No. 3, born 12th January was duly tested with tuberculin shortly after birth, and was fed from the milk of its own cow, and this animal failed to react when injected with tuberculin on 31st January and 6th July.

On 26th January, cow No. 7 calved, and its milk, subsequently retained for the use of its own calf, this animal tested with tuberculin on 31st January failed to react, and the same on 6th July.

On 31st January, and again on 6th July, these calves were injected with tuberculin, giving negative results. This interval of more than five months between the injecting of the tuberculin and their failure to react at the end of that period makes it reasonable to believe that so far these feeding experiments have demonstrated that the milk supplied, namely from cows, 1, 3, 5, 7, 9, and 10 has not proved infectious when fed to the calves.

It will thus be seen, that even in cow No. 1 the animal whose milk contained the largest number of bacilli, and that, especially of late months, in the greatest constancy, the effects of feeding calves was wholly without effect upon the animals, or in other words, evidently the number of the bacilli in this milk was below the minimum necessary to induce intestinal tuberculosis. In the healthy calf this absence of any reaction to the tuberculin test on the part of the calves was in harmony with the very small number of animals in which we were able to gain positive results upon inoculation and explains further the great difficulty we encountered in the earlier part of our investigations in detecting the bacilli in the milk.

AUTOPSIES PERFORMED UPON THE SUSPECTED ANIMALS.

The first of the animals to be killed was No. 9, killed on account of a broken limb, in the middle of March. Unfortunately, by some misunderstanding no word was given to us concerning the performance of the autopsy till after the event. In this animal, the glands around the base of the trachea were enlarged and definitely tuberculous with caseous foci breaking down and forming a cavity with cheesy walls; there was no sign of tuberculosis of the mammary glands. At most there was upon microscopical examination, evidence of inflammation of a catarrhal type in the larger tubes, but nothing more. The supramammary lymph glands situated to the front and above, were somewhat enlarged and to the naked eye appeared to be tuberculous, but under the microscope all there was to be made out was a simple chronic inflammation and some fibroid changes without a single tubercle.

Upon June 22nd cow No. 1 was killed and an autopsy immediately performed upon it. Here we will briefly point out that there was fairly extensive tuberculosis of both lungs, of the peribronchial and mediastinal glands, of the anterior retroperitoneal glands

as again of the supramammary lymph glands. The mammary glands themselves were free from naked eye or microscopical evidence of tuberculosis.

Upon August 5 cows 3 and 5 were killed and autopsies performed forthwith. Cow 3 was found apparently in excellent health. The udder showed no signs of disease upon the usual careful naked eye and microscopical examination. Elsewhere the only sign of tuberculosis was the presence of 7 or 8 tubercles in the peribronchial glands, as also to a still less extent, were tubercles rarely recognized in the mesenteric glands.

Cow 5 presented similar slight evidence of the disease. There were a few small scattered tubercles in the peribronchial lymph glands, together with definite evidence of tuberculosis, small in amount, affecting the liver, and a few tubercles in the mesenteric glands.

Upon September 7 cows 4, and 6 were killed. Cow 4 (one of the two animals it should be noted which had reacted to tuberculin in December, and had received later enormous doses of tuberculin without apparent effect) showed singularly little evidence of the disease. Scattered through the mesenteric glands were peculiar hardened encapsuled areas unlike anything seen in the other animals. Under the microscope there showed no tubercles but areas of fibroid deposit. It may legitimately be questioned whether these do not represent tubercle areas in process of absorption following upon the use of tuberculin. The mammary gland presented also some generalised fibrosis. The only evidence of the active disease was found in the small intestines. Here were occasional small ulcers $\frac{1}{2}$ to 1 cm. in diameter, which under the microscope were found to be typically tubercular. Their presence would almost seem to indicate the existence of other foci of tuberculosis in the mouth, pharynx, or upper portion of the digestive tract, or it might be in the larynx or other portions of the respiratory tract, but a most careful and full examination failed to discover such other foci.

Cow No. 6. This animal like cow No. 1 presented definite pulmonary tuberculosis in part breaking down. This was in the left lung. The right lung was free from disease. The peribronchial glands of the left side were somewhat enlarged with areas of caseous and calcified tuberculosis. There were no signs of tuberculosis elsewhere.

Upon October 12th the remaining cows were killed, namely Nos. 2, 8 and 10. Cow No. 2 showed pulmonary tuberculosis (apex of right lung), the condition being old and the nodules well encapsulated with fibrous tissue. The peribronchial glands of the same side were also affected, showing similar old fibrous tubercles.

Cow No. 8. Here the only evidences of tuberculosis discovered were in one of the peribronchial glands or more correctly in the large lymph gland situated at the bifurcation of the trachea. In this were three or four distinct tubercles the nature of which was confirmed by microscopical examination. Careful study and dissection of the other organs failed to show a single tuberculous focus.

Cow No. 10. The upper lobe of the right lung contained an extensive caseating area of tuberculosis about 5 by 2 inches in extent. In its neighbourhood were some disseminated smaller tubercles while the pleural surface presented some whitish tubercles the sides of grape seeds. No other of the series of animals presented such clear evidence of an advancing tuberculosis. Close to the bifurcation of the trachea there was extensive tuberculosis of the lymph glands with caseation, while the left lung contained in its lower lobe a smaller area of the disease. The udder was fibroid and presented what at first might be mistaken for caseous tuberculosis, but what upon microscopical examination were found to be numerous blocked and swollen lobules of the mammary gland filled with inspissated milk.

The following table gives the results of our examination in due order. It is to be understood that throughout we have under the microscope confirmed the diagnosis of tuberculosis made at the time of the autopsy.

ABSTRACT OF AUTOPSIES PERFORMED.

Cow No. 1.—Killed June 22nd. Fairly extensive tuberculosis of both lungs, of peribronchial and mediastinal glands, some tuberculosis of the retroperitoneal glands of the anterior or upper portion of the abdomen as again of the supramammary glands

Mammary glands free from naked eye or microscopical evidence of tuberculosis but somewhat fibroid.

Cow No. 2.—Killed October 12th. Upper half of right lung alone showed evidence of the disease. Here there was a small mass about 2 x 1 of discrete well encapsulated tubercular nodules with firm fibro-caseous contents. Peribronchial glands moderately enlarged with firm almost fibrous tubercles and in some of these slight calcareous change; peribronchial glands on the left side not enlarged, this was all the tuberculosis recognised. Mammary gland free from tuberculosis. A large retention cyst containing yellowish oily fluid in the left fore quarter.

Cow No. 3.—Killed August 5th. Animal apparently in good health; on removal of the udder, no evidence whatever of disease; glands in a soft healthy condition with complete absence of either fibroid or caseous change; both lungs normal. Peribronchial glands showed very slight increase in size and in them were recognized some 7 or 8 minute greyish nodules the size of peas. A few gray nodules not larger than peas in some of the mesenteric glands.

Cow No. 4.—Killed Sept. 7th. Udder found soft throughout, normal in size and colour without any evidence of fibroid increase or of tuberculosis; a few small retention cysts. Lungs and heart healthy. In small intestines at distant intervals were a few small ulcers from about $\frac{1}{2}$ cm. to 1 cm. ($\frac{1}{3}$ to $\frac{2}{5}$), which had a suspiciously tubercular appearance. Mesenteric glands not much enlarged, presenting peculiar hardened pigmented areas but no evidence of caseation. Microscopically, the ulcers were found to be truly tubercular, the mesenteric glands however showed nothing typically tubercular; there was some slight increase of fibrous tissue in the mammary gland but no evidence of caseation or necrosis; peribronchial glands quite normal.

In this case therefore the only definite tubercular lesions found were the ulcers in the intestine; the existence of these, however, should be regarded as evidence of tuberculosis elsewhere.

Cow No. 5.—Killed August 5th. Animal apparently healthy. Udder on examination showed total absence of tuberculosis and only in one portion was there slight fibrosis, evidently associated with the presence of same retention cysts. Lungs free from any evidence of tuberculosis; a few small tubercles in the peribronchial glands as again in the mesenteric glands; a single tubercular nodule (tubercular nature confirmed microscopically) in the liver with smaller caseating nodules, together with a subacute tubercular perihepatitis.

Cow No. 6.—Killed Sept. 7th. Udder showed no sign of tuberculosis; of good colour and consistency. No evidence of tuberculosis of abdominal organs; anterior border of lower portion of left lung showed a caseous partly liquefied tuberculous mass the size of a walnut, another mass the size of filbert or large hazelnut situated more centrally, was fibroid with dried caseous centre and no signs of breaking down, the rest of the lung seemed normal. No apparent lesion in right lung; peribronchial glands somewhat enlarged and with numerous caseous and calcified areas. Microscopically, tuberculosis and tubercle bacilli in the affected lung tissue and the peribronchial glands. The mammary gland presented no abnormality.

Cow No. 7.—Killed October 12th. Udder and supramammary glands perfectly healthy; no signs of abdominal tuberculosis, not a sign of tuberculosis anywhere in either lung; *the only evidence of the disease was a single small caseous mass* well encapsulated found in one of the peribronchial glands of the root of the left lung, the remaining peribronchial glands were quite healthy.

Cow No. 8.—Killed October 12th. Udder a little fibroid but no signs of tubercles; both supramammary glands quite healthy: no abdominal tuberculosis; both lungs apparently healthy. Here as in cows 7 and 9 the only evidence of tuberculosis discovered was in one of the peribronchial glands, this being the large one situated close to the bifurcation of the trachea.

Cow No. 9.—Killed March 15th. following an accident. No sign of tuberculosis of the mammary glands, but under the microscope, slight catarrhal inflammation

of the larger milk tubes; supramammary glands somewhat enlarged, microscopically, presenting simple chronic fibroid inflammation with no tubercles. Lungs free from tuberculosis; the glands at the bifurcation of the trachea were enlarged with distinctly tuberculous breaking down caseous foci.

Cow No. 10.—Killed October 12th. The right half of udder more especially showed frequent small whitish areas resembling cheesy tubercles and the supramammary glands appeared somewhat enlarged, but upon microscopical examination the suspicious areas in the udder were found to be throughout of the nature of small dilatations of lobules of the gland due to obstruction and these were filled with inspissated milk. The lymphatic glands were quite healthy. The upper lobe of the right lung contained an extensive caseating area of tuberculosis about 5" x 2". Within its neighbourhood, some disseminated smaller tubercles while the pleural surface presented some grape-seed whitish nodules or tubercles. Close to the bifurcation of the trachea, extensive caseous tuberculosis of the peribronchial glands; smaller area of tuberculosis in the lower lobe of left lung; no abdominal tuberculosis.

POST MORTEM UPON THE CALVES.

The calves were killed and examined upon October 12th and 14th respectively. Although careful examination was made not a single sign of tuberculosis was seen in any of the organs.

CONFIRMATION OF DIAGNOSIS MADE AT THE TIME OF AUTOPSY UPON THE COWS BY INOCULATION OF GUINEA-PIGS AND RABBITS.

To assure ourselves that the cattle still suffered from active tuberculosis, in connection with seven out of the ten animals we inoculated one or more guinea-pigs or rabbits with small pieces of tissues regarded by us as being tuberculous, the three exceptions being the animals killed on October 12th. In one case the assistant of the farm not having followed our directions the inoculations could only be carried out some four hours after the organs had been removed, and the weather being singularly hot putrefactive changes had already set in the affected glands so that the guinea-pigs died of septicæmia. With these exceptions all the inoculated guinea-pigs and rabbits have died of generalized tuberculosis. Here then is afforded an additional proof of the nature of the disease.

It is however worthy of remark that none of the animals so inoculated died under the average period. No death occurred within five weeks after inoculation; even in the case of Cow No. 1 the guinea-pigs (although relatively large portions of affected organs were inserted into the abdominal cavity) died in eight and nine weeks respectively. It is difficult to arrive at any other conclusion than that the bacilli present in these cattle were not particularly virulent.

STUDIES MADE UPON COW NO. I.

We think it well to give a more detailed description of our observations upon this animal and upon the infectiousness of its milk for reasons already stated, namely, that, as indicated by the frequent reaction to the tuberculin test, the presence of cough, the existence of tuberculous infection of the supramammary lymph glands and again by the frequent presence of the bacilli in the milk this animal evidently presented more advanced tuberculosis than did any of the others. This view was amply confirmed by the findings at the autopsy; and it is interesting therefore to examine in detail the results obtained even if in so doing we not infrequently have to recapitulate facts already brought forward in the previous portion of our report.

The animal was a full grown cow, a graded Ayrshire, which from all we can learn had been three years at the Experimental Farm at Ottawa, and there on November 18th, 1897, it had reacted definitely to tuberculin.

On examining the animal upon December 1st, at Outremont, the coat was found a little staring, the animal was spare without being emaciated and there was a cough of fair frequency. There was obscure dulness over the anterior portion of the left lung and above and in front of the udder on either side could be felt hard masses, that on the right side being the larger and being roughly, the size of a hen's egg. These two masses were firm and movable, and upon manual examination it was difficult to say whether they were within the gland substance or immediately in front of it. From their character as again from the general history of the animal, Professor McEachran, diagnosed these as being tubercular in nature.

At first the animal gave a fair though not an abundant supply of milk. Already by the 26th of December the milk obtained from the right anterior quarter diminished markedly in amount and became thin and watery containing numerous creamy flakes and as the largest tubercular mass was situated immediately over the quarter, special attention was paid to the milk from this region. Frequent examinations were made to detect bacilli in it, and 7 guinea pigs and 3 rabbits were inoculated with milk from this quarter alone.

As will be shown later on, no bacilli was found in this milk and not a single one of the inoculated animals showed a sign of tuberculosis. The condition of the right anterior quarter continued the same till the animal was killed in June and for the last five months the animal only gave from it from 2 to 4 ounces of thin fluid. The milk from the other quarters while continuing small in amount, was normal in appearance and properties.

The cough from which the animal suffered lessened in severity and by the end of January 18th, 1898, had ceased. From now on the condition of the animal remained very fair, there was no sign of further emaciation, the coat became better, and until its death, the animal continued to feed well and apparently was in a good condition, becoming evidently fatter. The condition of the udder was frequently noted and no evidence was found of the further enlargement of the masses recognised at the front of the udder nor did any new hard masses show themselves.

Upon June 22nd, the animal was led to the Montreal Hunt Club Grounds, a distance of about a mile; it was very active, and indeed lively upon this journey. Here it was poll-axed and an autopsy was performed by both of us, Mr. Brannen assisting.

There was abundant fat over the body, and the muscles and viscera were in general in a good condition. Upon opening the thorax, there was abundant evidence of the disease in the apical half of the upper lobe of the left lung. This apical half was transformed into large masses of tubercles which on the whole were sharply defined appearing to be of old standing, and there was a large tuberculous mass the size of a pigeon's egg having a caseous and breaking down central portion and the appearance of progressive ulcerative disease in its immediate neighbourhood. There were occasional tuberculous masses in other lobes of the left lung and more rarely in the right. There was no sign of grape tuberculosis of the pleuræ; there were however enlarged and tubercular peribronchial and mediastinal glands; these were in general firm and somewhat fibroid showing little evidence of caseation.

As a further evidence of the long continuation of the process, it may be remarked that slight grittiness was noted upon cutting open several of these, this grittiness being due to a deposit of calcareous salts. The heart and the pericardium were free from any signs of tuberculosis.

Abdomen.—All the large abdominal viscera were healthy and free from any sign of disease, but in the upper segment of the anterior half of the abdomen, especially in the neighbourhood of the stomachs, the abdominal lymphatic glands were enlarged, very firm, white and fibroid, some showed complete caseation of long standing, and all of these infected glands were so well encapsulated with fibrous tissue that the appearance given were those of an arrested tuberculosis of long standing. There was no other obvious tuberculosis in the abdominal cavity.

Udders.—The supramammary glands situated at the anterior extremity of either udder were very large, being about three inches long by two broad. Upon section, we found scattered throughout them fibroid tubercles which were especially well recognisable towards the periphery. The mammary gland substance itself was cut into small pieces and carefully examined. Despite this careful examination not a sign of ulcerative tuberculosis could be recognised; at most, the larger galactiferous ducts showed a rather suspicious appearance; occasionally there were minute fibroid masses raised above the general surface, presenting to some extent the characters of minute miliary tubercles but without any sign of ulceration.

Microscopical Examination.—Portions of tissue in the neighbourhood of the galactiferous ducts showed these apparent miliary tubercles under the naked eye, but when examined under the microscope showed no sign of tuberculosis; at most there was increased fibrous tissue and evidence of chronic simple inflammation. When stained to demonstrate tubercle bacilli, the sections gave throughout negative results. Very numerous sections taken from the mammary gland failed to reveal any tuberculosis. Here again there was general increased fibrosis of the gland as of old chronic inflammatory disturbance.

On the other hand, the supramammary lymphatic glands showed more especially in the periphery, well marked tubercles although with rare giant cells, and when properly stained the bacilli were discovered in these. No bacilli were found in the abdominal lymphatic glands, but the affected lung tissue and the peribronchial glands presented under the microscope all the appearances of a long continued tuberculosis with very little evidence in general of progressive disease, although as some of the masses were ulcerating, it was evident that the process was not fully arrested. The other organs of the body examined microscopically (lung, kidney, heart and spleen), showed no signs of tuberculosis.

It will be seen from the above account that the most extensive lesions in this animal were in connection with the lungs, the peribronchial and the mediastinal glands, and, upon the whole, the conclusion must be that the infection had originally started in the pulmonary area. Although the existence of old tuberculosis in the upper abdominal glands might possibly indicate that the first infection had been through the alimentary tract, and that the disease had extended upwards to the thorax along the thoracic duct, it is only in the lungs that there is any indication of progressive tuberculosis, and even there the extent was not so great as is frequently found, the main mass of the tubercles being well defined and encapsulated and in a condition of arrest.

Quite the most interesting appearance in this case is the definite tuberculosis of the supramammary glands without any associated evidence of tuberculosis of the mammary tissue itself. Two explanations immediately suggest themselves: either that at one period there had been an active tuberculosis of the mammary gland, and that the tubercle bacilli had passed along the lymphatic channels to the supramammary glands, and there led to the development of tubercles which had grown and persisted while the original slight tuberculosis in the mammary gland had been arrested and had undergone absorption; or, on the other hand, that the supramammary glands had become infected, not along the lymph channels from the gland, but through the blood stream. The fact that no other isolated lymph glands in other portions of the body were similarly affected, is against the latter supposition. The microscopical evidence of old chronic inflammation especially in the region of the galactiferous ducts, is to a certain extent, in favour of the former. But there is a possibility which must not be left out of account. It is quite possible, though we must confess unlikely that there were in the mammary gland some small foci of active tuberculosis which we failed to discover. We unlikely, because we made a full and careful study of the various portions of the gland. Again, it is possible that if the tubercle bacilli were brought to the gland by the blood stream and were excreted in the milk without setting up tuberculosis in the gland itself, some of these bacilli might, instead of passing out with the milk, be conveyed along the lymphatic channels to the supramammary glands and there, becoming arrested, might induce the development of tubercles. Of necessity, a complete explanation of this curious condition must be left open. We can only say

that we made a conscientious study of the gland tissue, and that, despite the discovery in the milk of bacilli, extending over several months, and despite the tuberculosis of the supramammary glands, we were wholly unable to discover any sign of active tuberculosis in the gland substance itself. And as a result of this failure to discover such active tuberculosis, we are inclined to range ourselves along with those who hold that tubercle bacilli may be excreted in the milk without there being developed or present an ulcerating tuberculosis of the mammary glands of teats. We may add that in the other cows in which we occasionally come across the bacilli in the milk in small numbers, we also failed to detect active tuberculosis of the mammary glands.

Examination of the milk.—During the latter half of December, 1897, and the beginning of January 1898, the morning milk of twenty successive days from cow No. 1, was subjected to bacteriological examination in order to detect tubercle bacilli, both that drawn from all four quarters and then mixed, and that from the right anterior quarter alone. At first the milk was allowed to stand for two days in tall bottles with conical bottoms in order to sediment bacteria and other more solid particles; this sediment was then examined by the method already stated and no tubercle bacilli were found by this means either in the whole milk or in that from the anterior quarter. In that from the anterior quarter alone, there were abundant diplococcus forms. In this series of milks to which no antiseptic had been added, the abundance of extraneous bacteria added to the difficulties in the search. We very soon added, therefore, as soon as the milk came in, 5 per cent of glacial carbolic acid subsequently reduced to $2\frac{1}{2}$ per cent. This addition of carbolic acid has been found in the case of the sputum for example, to aid in the ease and certainty of staining by the carbol fuchsin method. Carbolised milk from cow No. 1, thus left to stand and precipitate sediment gave also negative results.

In January, under our direction, Mr. Hammond made a series of investigations to see whether bacilli if present would be more certainly brought down in the process of coagulating the casein in the milk, by the addition of acetic acid. Coagulum, so formed, when smeared over the surface of a series of slides and stained in the usual manner, gave no clear results, but when this coagulum was further hardened in alcohol, imbedded in celloidin and then cut upon the microtome, in two of the sections so made, tubercle bacilli were recognised.

Up to this point then, it was evident that if the milk contained the tubercle bacilli, it was in very small numbers, for other observers have by such methods frequently detected the bacilli. Some method was necessary which should concentrate these to the greatest possible extent and render them recognisable; that method, as already stated, is a modification of ordinary centrifugalisation. 30 cc. or about 2 oz. of milk were taken and placed from 20 minutes to half an hour in Purdy's Electrical Centrifuge, giving from 3000 to 5000 revolutions per minute, the supernatant fluid was decanted off and a few drops of caustic potash were added to the sediment in order to dissolve out proteid and fatty matters. Distilled water was now added in order to dilute and to a large extent remove the caustic potash and the suspension was again centrifugalised, the supernatant fluid poured off and the sediment smeared over two slides, fixed and stained by Gabbett's method. By this means we at first irregularly, but later in May and June constantly, obtained the tubercle bacilli in the total milk from this cow No. 1 although we never obtained them from the small amount of serous fluid gained from the right anterior quarter. Counting the total number thus obtained from 30 cc. of the milk of cow No. 1, the highest number that we could ever find was from 40 to 50 more frequently especially during January and February our preparations showed but three or four or five which we could venture to state were true tubercle bacilli.

Now it is interesting to note that according to Wgssokowicz, there is a minimal number of tubercle bacilli which have to be inoculated into an animal in order to induce the disease. A single tubercle bacillus inoculated into the peritoneal cavity is destroyed, and he concluded that more than 15 must be given to a guinea pig for the disease to be set up in that animal. From 20 to 30 cc. of milk is the amount (and that a large one) which we employed for our purposes of inoculation. Granting then that the milk contained tubercle bacilli, and if Wgssokowicz be correct (and his observations are in accord with the observations made by others in connection with other bacteria,) then in genera

especially during the earlier months, we inoculated too few bacilli to have any effect. In other words, the milk of the tuberculous cow, while it contains tubercle bacilli, may still be harmless to the more susceptible rabbit and guinea-pig, the number of bacilli present being too few. If thus harmless to these animals it would be harmless to human beings when taken by the mouth, for in the first place the human being would appear not to be so susceptible to tuberculosis as is the rabbit or the guinea-pig; and in the second place, it is well established that inoculation of bacteria direct into the peritoneal cavity, is a more certain method of inducing disease than mere swallowing and ingestion by the alimentary tract. As our observations further show, the long continued feeding of this milk to a calf was without result. This helps to explain how it is that milk from cows known to be tuberculous may often be consumed for long periods, even by young children with apparent impunity. In the earlier portion of this report we have pointed out how, notwithstanding these considerations, such milk must be condemned.

A further interesting point is that this method employed by us for detecting the bacilli in milk, is a more sure means of determining their existence in the same when present in small quantities, than is the method of intraperitoneal inoculation into the rabbit or guinea-pig.*

THE INOCULATIONS OF THE TISSUES OF COW NO 1, INTO GUINEA PIGS.

Two guinea-pigs were inoculated on June 22nd, intraperitoneally, with small portions taken from the tuberculous masses in the lung of this cow No 1. Of these one died seven weeks later, upon August 3rd, and on examination it was found to be greatly emaciated. Upon cutting through the abdominal wall there were frequent subperitoneal tubercles in the neighbourhood of the operation wound, and the portion of tissue inoculated was found bound up in the omental folds which were also the seat of tuberculosis. The mesenteric and retroperitoneal glands were greatly enlarged and there was a large clump of the affected glands near the region of the cæcum. The spleen contained numerous relatively large tubercles scattered throughout its substance, the liver was large and mottled with numerous less well-defined tubercles, the kidneys and suprarenals presented no obvious tuberculosis; there was extensive sero-fibrinous tuberculous pleurisy with minute miliary subserous tubercles scattered over the surface of the lungs; tubercles of mediastinal glands and enlargement of the cervical glands.

Microscopical examination showed abundant tuberculosis, in the liver as again in the spleen and rare minute tubercles in the kidneys.

The other guinea-pig died on the 9th of August and showed a very similar distribution of tuberculosis; there was more extensive tuberculosis of the liver and the process also extended to the pleural cavity and the lungs.

It is interesting to note here that although relatively large portions of the lung tissue had been introduced into the peritoneal cavity, death occurred at a somewhat later period than is usual in virulent tuberculosis.

REACTION TO TUBERCULIN, COW NO. 1.

In the previous portion of the report we have already referred at some little length to the reaction of this cow, we need here therefore but briefly summarise the facts.

The animal reacted at Ottawa on November 5th, gave a pronounced reaction again, 42 days later at Outremont on December 17th giving a rise of 4.7° ; it did not react to the attenuated tuberculin on January 5th; on January 15th, 29 days after the last proper injection there was however a well marked rise of 4° .

* In making this statement it must be kept in mind:—

- i. That the animals studied by us were free from mammary tuberculosis.
- ii. That the bacilli present in the milk would seem to have been excreted and to be of lessened virulence.
- iii. That where there is active tuberculosis of the udder and virulent bacilli pass directly into the milk it is probable that the inoculation test is the more certain.

As already stated it is interesting to note that in this animal reaction was obtained within 30 days although it has been laid down by some workers that within this period no reaction is to be obtained in the cow.

THE RESULTS OF INOCULATIONS FROM COW NO. 1.

Leaving out of consideration certain control animals, we inoculated 29 guinea-pigs and 26 rabbits. Of these, as above mentioned, 7 guinea pigs and 3 rabbits were treated with the milk from the right anterior quarter of the udder alone, the rest from the mixed milk from the three or four quarters or from products of the same. The inoculations were, throughout, intraperitoneal, milk being introduced by a syringe through the abdominal wall into the peritoneal cavity. The amounts of milk or "milk products" varied in the guinea-pig from 2 cc. of the centrifugalised sediment (obtained from 15 cc. of the milk) to 35 ccm. of the fresh milk, the average and most usual amount being 20 ccm. of fresh milk, or about two-thirds of an ounce. In the rabbit from 10 to 60 ccm. of the fresh milk was used, the average being 25 ccm.

Of all these animals so treated only two guinea-pigs developed tuberculosis, or 6·8% of the inoculated guinea-pigs became affected. These figures require a further correction, three of the guinea pigs having died from sepsis or other cause within three weeks, *i.e.*, before peritoneal tuberculosis could surely manifest itself. It is thus correct to state that two out of 26 guinea-pigs succumbed to tuberculosis induced by milk from cow No. 1, or 7·7 per cent.

Similarly, one rabbit succumbed to septic infection and must be left out of consideration. It has thus to be stated that out of 25 rabbits, not a single one died from tuberculosis as the result of inoculation with the milk of this cow.

Here too must be mentioned the attempt to convey the disease from Cow 1 to a calf by means of feeding that calf solely with the milk. For five months the calf was so fed, and at the end of this time it was apparently in excellent health; it failed to react to tuberculin, and when four months later it was killed there was not a trace of tuberculosis anywhere throughout its tissues.

These results must not be considered unsatisfactory, the most that they prove is that unless very large numbers of animals be inoculated, the inoculation test, highly as many observers have regarded it, cannot be considered by any means a sure method for the detection of tuberculosis. Clearly, as already suggested, the milk of cow No. 1 contained in general a number of bacilli below the minimum necessary to infect the guinea-pig. Here we should add that the rabbit is not so susceptible to tuberculosis as the guinea-pig nor the calf so susceptible as the rabbit, while intraperitoneal inoculation is as a rule much more fatal than feeding with bacilli.

Careful centrifugalisation and the use of a satisfactory method for the removal of proteid and fatty matters has in our hands given much more sure results; but we have to admit, taking our other cows into consideration, that bacteriological examination of the milk will not always indicate the existence of tuberculosis. We thus, from a study of these cows from the Experimental Farm, can but fall back upon the now well established conclusion that of all the methods devised for the detection of the tuberculosis in cattle, none approach in sureness and value to the Tuberculin Test.

SUMMARY AND CONCLUSIONS.

The following are the main conclusions reached by us in the course of our observations upon the cattle from the Experimental Farm at Ottawa, studied by us at Outremont.

1. Without exception all the ten cows which had reacted to tuberculosis at Ottawa presented upon post-mortem examination, seven months later, distinct evidences of tuberculosis.

2. In not one of the animals was the disease generalized or very extensive. In only four out of the ten was there pulmonary tuberculosis. In nine out of the ten, however, there was distinct evidence of the disease in the peribronchial and peritracheal lymph glands, indicating infection through the respiratory tract. In one, the only positive sign of active disease found was the presence of tubercular ulcers in the small intestines. In not one, despite most careful examination, could tubercles be detected in the mammary glands, although in Cow No. 1 (the animal presenting a more extensive tuberculosis than did any of the other animals) tubercles were discovered in the supra-mammary lymph glands. In three animals the disease appeared to be confined to the peribronchial glands, being found there in a latent condition. It would in fact be difficult to have a smaller amount of tuberculosis present than was found in these three.

3. Clearly therefore, as noted by previous observers, the first injection of tuberculin is capable of detecting the slightest degree of infection in cattle.

4. We confirm the observations of previous workers in finding that one injection of tuberculin in cows seriously affects the development of the reaction within the next few weeks. This inhibitory effect may last for considerably over thirty days. But in one case we obtained a well marked reaction within thirty days.

5. It would seem from our observations that a second reaction is not more easily obtained within thirty days by doubling or quadrupling the dose of tuberculin. Herein the cow would seem to differ from man.

6. It is not to be left out of account that possibly, repeated doses of tuberculin, in animals slightly affected, have a certain curative power. In this way, may, possibly, be explained the singularly slight evidences of the disease found in certain of the animals, more especially in those subjected to repeated large doses.

7. While thus these tuberculous animals were free from tubercular disease of the udders, the milk of several contained from time to time tubercle bacilli. The only satisfactory explanation of their presence is that the mammary gland possesses the power of removing pathogenic organisms from the blood and lymph and of excreting them in a condition of lessened virulence;

8. That the bacilli discovered by us in the milk were truly tubercle bacilli is supported by the following facts:—

(a.) The bacilli were found in greatest number and most frequently in the milk of those animals which, as shown by the autopsy, presented the most extensive evidence of the disease.

(b.) Two guinea-pigs and one rabbit inoculated with such milk died of generalised tuberculosis.

(c.) The staining reactions of the bacilli were those peculiar to the Tubercle bacilli.

(9.) On the other hand, it is clear that the milk of animals suffering from these slight grades of tuberculosis, and free from tubercular disease of the mammary glands *in general* possesses very feeble infective powers.

(a.) When the milk was injected in large quantities into the abdominal cavity of that most susceptible animal, the guinea-pig, *i. e.* when the most favourable method is employed to reproduce the disease, out of 44 guinea-pigs thus inoculated only two succumbed to the disease. Of 42 rabbits similarly inoculated only one became infected.

(b.) Young calves fed entirely upon the milk of these infected cows over a period of several months remained wholly free from the disease, did not react to the tuberculin test and upon post mortem examination showed not the faintest trace of tuberculosis despite most careful search.

10. But, as shown by a study of the milk, *occasionally without obvious cause the number of bacilli present in it might increase greatly* and in one case the number suddenly became so great that the milk must have been at this period a most dangerous food stuff.

11. Throughout the whole period during which these cows were under observation only one—and that one only at the beginning—gave strong clinical evidence of the existence of the disease.

What practical conclusions are to be drawn from the results here set forth?

Remembering always that the cattle in question were animals that had been well cared for and that had, without exception, only relatively slight traces of the disease and no signs of tuberculosis of the udder, it may be acknowledged that these studies demonstrate that the infectivity of such animals (especially by the milk) is very slight. We have, it will be seen, made no studies as to their capacity to infect other cattle by the breath or discharge from the respiratory passages. The findings at the post mortem led us to conclude that in only four of the animals could there possibly have been infections conveyed to others through these channels.

We are forced then to conclude that animals like these, presenting no clinical symptoms and free from mammary tuberculosis are relatively harmless so long as they continue in such a state. What position it is certain to be asked, should the private individual and the government take in relation to animals of this character?

This question it is not wholly easy to answer. Our first consideration must be that tuberculosis is an infectious disease, and, as such, it is the duty of the individual, as of the government, to stamp it out from among our herds at all costs. We have abundant examples here in Canada, as elsewhere throughout the civilized world, that the introduction of a bull or other animal with advanced tuberculosis into a herd, or into a district, has led to the spread of the disease, to the destruction of valuable herds and to almost incalculable loss to the farmer. Our observations do not in the slightest affect these well attested facts. Like any other infectious disease tuberculosis must be stamped out. If it be not stamped out then it can be stated with absolute conviction that just as hogs with "healed" hog cholera may be the means of eventually infecting the piggeries into which they are introduced, or as cattle with old encapsulated pleuropneumonia may similarly cause a reemergence of the disease, so certain of the cattle which have reacted to tuberculin, although free from the clinical symptoms of the disease, if kept upon the farm will inevitably, sooner or later, develop a dangerous and distinctly infective condition. It is imperative, therefore, that such cattle be either destroyed or segregated and prevented from spreading the condition.

We must admit that it appears a severe measure to urge the destruction of cattle which if kept under healthy conditions would for a long period be capable of putting on flesh and of calving, animals, that is to say, which are not only of present but of prospective commercial value. But is the alternative course, of segregation, feasible? In the case of farmers having valuable herds and prepared to submit to the visits and rulings of the government Inspector it would be policy to advise the erection of a separate byre and the fencing off of a special pastures for animals reacting to tuberculin but presenting no clinical symptoms. The cows could well be employed for breeding purposes. Many farmers could not afford the expense of separate buildings and separate attendance, while with both classes of farmers there would be a strong temptation to utilise the milk when that milk ought assuredly to be condemned on account of possible danger.

In Germany the Government undertakes the sale of the meat of condemned animals, cooked or uncooked, according to the extent of the disease, and in this way the animals are far from being a total loss. Here in Canada we have not the large poor population willing to buy such meat, nor again would it be possible for the government to go to the expense of establishing segregation farms in the different districts. The country is too large and again there would be hostility towards purchasing calves, etc. from an establishment of this nature. The question of cost would alone defeat this scheme. Yet granting everything we have to admit that the animals under discussion are of distinct value and that this report is likely to raise the question of compensation. It is not for us to gloss over the findings of this report. They must stand upon their merits. If we be not venturing too far outside our province we would respectfully suggest that admitting that Tuberculosis among cattle is an infectious—and therefore, a preventable—disease, it is the duty of the government to see that it is minimised as much as possible.

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